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Characterization of a triple-recombinant, reassortant rotavirus strain from the Dominican Republic

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Abstract

We report the genome of a novel human triple-recombinant G4P[6–8_R] mono-reassortant strain identified in a stool sample from the Dominican Republic during routine facility-based rotavirus strain surveillance. The strain was designated as RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R], with a genomic constellation of G4-P[6–8_R]-I1-R1-C1-M1-(A1-A8_R)-N1-(T1-T7_R)-E1-H1. Recombinant gene segments NSP1 and NSP3 were generated as a result of recombination between genogroup 1 rotavirus A1 human strain and a genotype A8 porcine strain and between genogroup 1 rotavirus T1 human strain and a genotype T7 bovine strain, respectively. Analyses of the RNA secondary structures of gene segment VP4, NSP1 and NSP3 showed that all the recombinant regions appear to start in a loop (single-stranded) region and terminate in a stem (double-stranded) structure. Also, the VP7 gene occupied lineage VII within the G4 genotypes consisting of mostly porcine or porcine-like G4 strains, suggesting the occurrence of reassortment. The remaining gene segments clustered phylogenetically with genogroup 1 strains. This exchange of whole or partial genetic materials between rotaviruses by recombination and reassortment contributes directly to their diversification, adaptation and evolution.

Keywords

rotavirus; triple recombinant; reassortment

Introduction

Group A rotaviruses (RVAs) are a widespread pathogen associated with mild to severe gastroenteritis and are a major cause of morbidity and mortality in young children and

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The GenBank/EMBL/DDBJ accession number for VP1, VP2, VP3, VP4, NSP1, VP6, NSP3, NSP2, VP7, NSP4 and NSP5 is KX778612–KX778622, respectively.

Two supplementary figures are available with the online Supplementary Material.

animals [1]. Recent figures revealed an estimated 215 000 preventable paediatric fatalities in low-income countries of Asia and Africa are attributable to rotavirus group A [2]. In the Dominican Republic, an estimated 425 preventable deaths among children aged 5 years and younger are attributed annually to rotavirus infections [3].

Rotavirus, a genus of the *Reoviridae* family, contains a genome of 11 segments of double-stranded RNA coding six structural and five or six non-structural proteins [1]. Given the segmented nature of the rotavirus genome, the genes encoding each segment can undergo reassortment *in vivo* after co-infection with rotavirus strains belonging to the same group or species [4–6].

The classification nomenclature for the genes of RVA is Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, with x indicating the numbers of the corresponding genotypes [7]. To date, there are 32 G, 47 P, 24 I, 18 R, 17 C, 17 M, 28 A, 18 n, 19 T, 24 E and 19 h genotypes recognized among RVAs in humans and animals (<http://rega.kuleuven.be/cev/viralmetagénomics/virus-classification>). The majority of human RVA strains possess either the Wa-like genogroup 1 constellation (Gx-P[x]-I1-R1-C1-M1-A1-N1-T1-E1-H1), which contains mostly strains of porcine origin, or the DS-1-like genogroup 2 constellation (Gx-P[x]-I2-R2-C2-M2-A2-N2-T2-E2-H2), which consists of strains typically of bovine origin. Occasionally, human RVA strains have the AU-1-like genogroup 3 constellation (Gx-P[x]-I3-R3-C3-M3-A3-N3-T3-E3-H3) that comprised strains mostly of feline origin [1, 8].

Expansion of the rotavirus classification system to include all 11 genome segments of rotavirus [8] has extended our knowledge on the ubiquitous nature and diversity of rotaviruses and the mechanisms involved in their evolution [6, 8]. These mechanisms include accumulation of point mutations, gene rearrangement (e.g. deletions, duplications and insertions), genomic reassortment [1, 6] and genetic recombination [9]. The most essential requirement for the last two mechanisms to occur is mixed infections with two different strains in one individual [10]. Each of these aforementioned mechanisms plays a vital role in the diversity, evolution and adaptation of rotavirus [11]. Though rare amongst RVAs, recombination events have been reported for gene segments VP1–VP4, VP6–VP7 and NSP1–NSP5 [9, 12–18]. The occurrence of multiple recombination events in a reassortant rotavirus strain has not been observed previously, however. Here, we present full genome characterization of an unusual human triple-recombinant, reassortant strain from the Dominican Republic.

Results

Genome constellation of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R]

The complete ORF sequences for all 11 genome segments of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] were deposited in GenBank under accession numbers KX778612 to KX778622 for VP1, VP2, VP3, VP4, NSP1, VP6, NSP3, NSP2, VP7, NSP4 and NSP5, respectively. Compared to the strains in GenBank, the nucleotide identities of all 11 genome segments of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] were at least 4 to 17% above established genotype cut-off values [8] (Table 1). The nucleotide and amino acid identities of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R],

compared to strains available in GenBank, indicated that 7 of 11 genes were closely related (nucleotide, 96.8–99.9 % and amino acid, 98.5–100 %) to rotavirus strains of human origin. The VP7 gene was closely related to VP7 genes of porcine origin. The remaining three gene segments, VP4, NSP1 and NSP3, were moderately related to similar gene segments of human origin, sharing nucleotide and amino acid homologies in the range of 89.1–91.4% and 89.7–94.2%, respectively. The full genomic constellation of the strain was determined to be G4-P[6–8_R]-I1-R1-C1-M1-(A1-A8_R)-N1-(T1-T7_R)-E1-H1 with ‘X-X_R’ denoting recombinant genes (see section below).

Sequence analyses of gene segment VP7

Phylogenetic analysis of the VP7 gene (Fig. 1a) indicated that strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] grouped into a sublineage within lineage VII [19], which comprised porcine strains HLJkd from China, Ro1 from India, ICB2185 from Brazil and P30 from Argentina. Strains in this lineage shared moderate nucleotide and amino acid identities in the range of 85.8–91.5% and 91.7–93.9 %, respectively.

Sequence, recombination and secondary structure analyses of gene segments VP4, NSP1 and NSP3

Although the nucleotide (range: 81.6–91.9 %) and amino acid (range: 89.7–94.8%) identities of the VP4, NSP1 and NSP3 gene segments showed moderately close relationships with genogroup 1 strains belonging to genotypes P[8], A1 and T1, respectively, phylogenetically, they did not cluster into distinct lineages, but fell between genotypes P[6] and P[8] (VP4), A1 and A8 (NSP1) and T1 and T7 (NSP3) in their respective phylogenies (Fig. 1b–d).

For VP4 gene segment, recombination analysis identified breakpoints at positions 228 and 1685 within this VP4 sequence using all six recombination detection methods embedded in RDP4 and with significant *P*-values (Fig. 2a). In regions 228–778 and 1685–1866, strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] shared 92.5–93.6% nucleotide homology with strain RVA/Human-tc/CHN/R479/2004/G4P[6] (GU189554; minor parent), respectively. However, in regions 9–227, 779–1684 and 1867–2328, the VP4 gene of strain 2013840364/G4P[6–8_R] exhibited a high level of similarity to strain RVA/Human-tc/E1911/2009/G1P[8] (JQ087448; major parent) in the range of 96.9–98.7%.

For the NSP1 gene, recombination analysis identified three potential recombination breakpoints with the highest degree of confidence at nucleotide positions 379, 503 and 1152 as shown in Fig. 2(b). As illustrated in this figure, all recombination events are between the genogroup A1 NSP1 genotype represented by human strain B3458/G9P[8] (EF990709) detected in Belgium in 2003, as the major parent, and genotype A8 porcine strain CMP45/G9P[20] (AB779636) detected in Thailand in 2008, as the minor parent, which led to the recombinant NSP1 gene of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R]. In these events, B3458/G9P[8]-like strain exchanged regions 379–502 and 1152–1458 of the NSP1 gene with a porcine-like strain (CMP45/G9P[20]) (Fig. 2b). From nucleotide positions 379 to 502, strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] shared 93.1 % nucleotide identity with strain CMP45/G9P[20], and these two strains clustered together in the tree (Fig. 2b). In regions 1152–1458, strain RVA/Human-wt/DOM/2013840364/2013/

G4P[6–8_R] shared a nucleotide identity of 99.2% with porcine strain CMP45/G9P[20]. In the 5' regions 32–378 and regions 503 to 1151, strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] exhibited a high level of similarity (99.2%) to the NSP1 gene from B3458/G9P[8], and these strains grouped together in the phylogenetic tree (Fig. 2b).

For the NSP3 gene, recombination was identified at nucleotide position 400 of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R], which was indicated as a recombinant of Bangladesh genogroup 1 human strain Dhaka25/G12P[8] (DQ146657) as major parent and genotype T7 bovine strain RVA/Cow-tc/GBR/UK/1973/G6P7[5] (K02170) as minor parent. The NSP3 sequence of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] clustered in different branches when different regions of the ORF (regions 25–399, 400–806 and 807–933) were used, indicating evidence of recombination (Fig. 2c).

Further analyses of the RNA structures of genes VP4, NSP1 and NSP3 showed several stable and unstable secondary structures (hairpins) just upstream of each crossover site. Also, recombination occurred primarily in predicted single-stranded regions and terminated in double-stranded regions (Fig. S2, available in the online Supplementary Material). The optimal free energy (ΔG) of the most stable RNA structure for the VP4, NSP1 and NSP3 gene sequences were -466.20 , -282 and -183 kcal mol $^{-1}$, respectively.

Sequence analyses of genes VP1–VP3, VP6, NSP2, NSP4 and NSP5

The VP1, VP2, VP3, VP6, NSP2, NSP4 and NSP5 gene segments of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] shared high nucleotide (96.8–99.9%) and amino acid (99.4–100%) identities with strains RVA/Human/NCA/64J/2010/G3P8 from Nicaragua, RVA/Human-wt/CHN/BJ-CR5317/2008/G9P[8] from China, RVA/Human-wt/ITA/JES11/2010/G9P[8] from Italy, RVA/Human-wt/BGD/Dhaka25/2002/G12P[8] from Bangladesh and RVA/Human-wt/BEL/B4633/2003/G12P[8] from Belgium (Table 1). Further analyses showed that the VP1–VP3, VP6, NSP2, NSP4 and NSP5 gene sequences of the Dominican Republic strain clustered phylogenetically to strains in the R1, C1, M1, I1, N1, E1 and H1 genotypes, respectively (Fig. S1).

Discussion

Here, we report the first full-length genomic rotavirus RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] sequence data from the Dominican Republic, with evidence that a single segment reassortment and multiple intergenotype recombination events have occurred.

Analysis of the complete ORF of each gene of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] revealed evidence of (1) intergenotype recombination with a double breakpoint in the VP4 gene, with a genogroup 1 P[8] strain (E1911/G1P[8]) as major parent and a P[6] strain (R479/G4P[6]) as minor parent, (2) intergenotype recombination with a triple breakpoint in the NSP1 gene, with two wild-type strains, B3458/G9P[8] of human origin (major parent) and CMP45/G9P[20] of porcine origin (minor parent), and (3) an intergenotype recombination with a single breakpoint in the NSP3 gene, with Dhak25/

G12P[8]-like human strain (major parent) and UK/G6P7[5]-like bovine strain (minor parent).

Infection of a single host cell by different or heterotypic rota-virus strains is a prerequisite for recombination events to occur in nature [14, 16, 18, 20, 21]. Recently, a number of reports have documented intragenic, intergenotype, interline-age, intersublineage and intersegmental recombination events within all six rotavirus structural and five non-structural genome segments [9, 12–18]. In 2011, Jere and colleagues reported evidence of multiple recombination involving genes VP6, NSP2 and NSP3 of rotavirus strain RVA/Human-wt/ZAF/2371WC/2008/G9P[8] [14]. This study depicts an active or ongoing occurrence of recombination since multiple rotavirus strains were detected in the stool sample. However, in the present study, only a single strain of rotavirus with multiple recombinant genes was detected in the stool sample, suggesting that the process of recombination occurred in another host and was transmitted to the child.

Despite all the reports of recombination events in rotaviruses, there are no established mechanisms on how this process of recombinant formation occurs. The replicative, copy-choice model, which proposes that the viral polymerase changes templates during synthesis of the nascent strand, producing a chimeric genome that contains fragments of both parental templates, has been proposed as the major molecular mechanism for both segmented and non-segmented RNA viruses [22–29]. Several reports have indicated that for copy-choice mechanism to work successfully, a stable secondary structure or hairpin and short stretches of AU-rich sequences must be present [24, 26, 30, 31]. We propose a stepwise copy-choice model by which the rotavirus strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] identified in this study was generated. In this study, the presence of several stable and unstable secondary structures detected just upstream of the various crossover sites in the recombinant gene segments VP4, NSP1 and NSP3 and the short stretches of the AU-rich sequences in between recombination breakpoints may be associated with the formation of this chimaeric sequence. Furthermore, all the recombinant regions appear to start in a loop (single-stranded) region and terminate in a stem (double-stranded) structure (Fig. S1).

The complete ORF for all 11 genes revealed that this Dominican Republic strain, designated RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R], possesses a porcine-like VP7 (G) and human-like VP4 (P) gene combination of G4P[6–8_R] and a full genome constellation of G4-P[6–8_R]-I1-R1-C1-M1-(A1-A8_R)-N1-(T1-T7_R)-E1-H1. The G/P combination of G4P[6–8_R] on entirely a genogroup 1 genetic backbone showed that this strain was formed though a single genetic reassortment event in which a G4P[8]-like strain with a typical genogroup 1 background acquired the neutralization antigen (VP7) from a porcine-like G4 strain that most likely circulated locally. This evidence is supported by the fact that the VP7 gene of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] clustered with porcine and porcine-like human rotaviruses in lineage VII [19]. Also, with the exception of the recombinant VP4, NSP1 and NSP3 gene segments, the remaining seven gene segments (VP1-VP3, VP6, NSP2, NSP4 and NSP5) clustered with rotaviruses of human origins belonging to cognate gene sequences of genogroup 1 genotypes.

This report provides direct evidence of recombination in both the structural protein-coding (VP4) as well as the non-structural protein-coding (NSP1 and NSP3) regions of the rotavirus group A, strain RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] involving two heterogenic parents in each case. Also, exchange of different genomic regions or whole-genome segment between rotaviruses by recombination and reassortment contributes directly to their diversification, adaptation and evolution. Finally, recombination events, though rare, if not detected, can have a huge impact on the accuracy of reported data especially on rotavirus surveillance studies.

Methods

Stool sample, RNA extraction, genotyping and sequencing of segment 4 (VP4) and 9 (VP7) genes

The stool sample was collected in 2012 from Hospital Infantil Dr. Robert Reíd Cabral, Dominican Republic, as part of a facility-based rotavirus strain surveillance system. The sample was from a 16-month-old male child with severe case of diarrhoea (four episodes per day), vomiting (five episodes per day) and fever with a maximum temperature of 40 °C. The sample was forwarded to the Centers for Disease Control and Prevention for routine rotavirus strain genotyping.

Rotavirus dsRNA for routine VP4 (P) and VP7 (G) genotyping was extracted from the sample using the MagNA pure compact RNA extraction kit on the MagNA Pure Compact instrument (Roche Applied Science) following the manufacturer's instructions. The extracted dsRNA was denatured at 97°C for 5 min and RT-PCR using the Qiagen OneStep RT-PCR kit (Qiagen) and DNA cycle sequencing was carried out as previously described [20, 32, 33]. Sequence chromatogram files were edited and sequence contigs were assembled using Sequencher 5.0 software (Gene Codes Corporation). Nucleotide similarity searches were performed using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to query the GenBank sequence database. Genotypes were determined using the RotaC online classification tool (<http://rotac.regatools.be/>) [34].

Rotavirus dsRNA extraction, purification, cDNA synthesis and amplification, and sequencing of rotavirus complete genome

Rotavirus dsRNA for full genome sequencing was extracted from the faecal sample following a previously described method [35]. The sequencing templates were prepared by using sequence-independent whole-genome reverse transcription PCR amplification [14, 35]. The amplified cDNA amplicons were sequenced using the Illumina MiSeq reagent kit v. 2, 500 cycles and the standard 250 bp paired-end reads method. Confirmations of next generation sequence results for VP4, NSP1 and NSP3 genes were done by Sanger sequencing. In brief, previously published consensus primers specific for each of these three genes were used to generate amplicons and then sequenced as described by Matthijnssens and colleagues [36].

Sequencing data analysis

Illumina sequence reads were analysed by using CLC Genomics Workbench 7.0.4 software (<http://www.clcbio.com/products/clc-genomics-workbench/>). A combination of *de novo* assembly followed by mapping to G4P[8] and G4P[6] reference strains from both human and animal were used to obtain the full-length genome of strain 2013840364. For each gene, multiple alignments were made by using the MUSCLE algorithm implemented in MEGA6 [37]. Once aligned, ML trees were constructed for each genome segment in PhyML 3.0 (<http://www.atgc-montpellier.fr/phyml/>) by using the optimal model for each alignment as identified by jModeltest 2.0.2 [38] and approximate-likelihood ratio test statistics computed for branch support [39]. For each genome segment, the models based on Corrected Akaike Information Criterion, VP1 (TrN+I), VP2 (TIM1+I), VP3 (TPM1uf+I), VP4 (GTR+I+G), VP6 (TPM3uf+I), VP7 (TIM2+I+G), NSP1 (GTR+I), NSP2 (TrN+I), NSP3 (HKY+I), NSP4 (HKY +G) and NSP5 (TPM2uf+I), were found to be the best fit for the sequence data. Nucleotide distance matrices were prepared using the *p*-distance algorithm of MEGA6 software [37].

Recombination and secondary structure analyses

Genes that did not cluster with a single genotype but rather between two genotypes suggesting partial sequence similarity with both types were subjected to recombination analyses. This was carried out using seven methods (RDP, GENECONV, MaxChi, BootScan, Chimaera, SiScan and 3Seq) implemented in RDP4 version 4.46 [40, 41] using default parameters for each method. Only recombination events detectable by two or more methods were considered evidence of recombination. Potential recombinant sequence, candidate parental sequences as well as possible recombination breakpoints were further confirmed using Simplot 3.5.1 [42] and Genetic Algorithm Recombination Detection (GARD) method embedded in the Datamonkey webserver [43, 44]. To confirm the RDP4, Simplot and GARD results, the sequences were split at the suggested recombination site(s) and separate ML nucleotide phylogenetic trees were constructed using MEGA version 6 software [37].

The DNafold (Vienna tools) embedded in the Geneious software version 8.1.8 (www.geneious.com) [45] was used to determine RNA structures using the default parameters. DNafold was also used to measure the optimal free energy (ΔG) for each recombinant gene sequence using its default parameters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation

RVA group A rotavirus

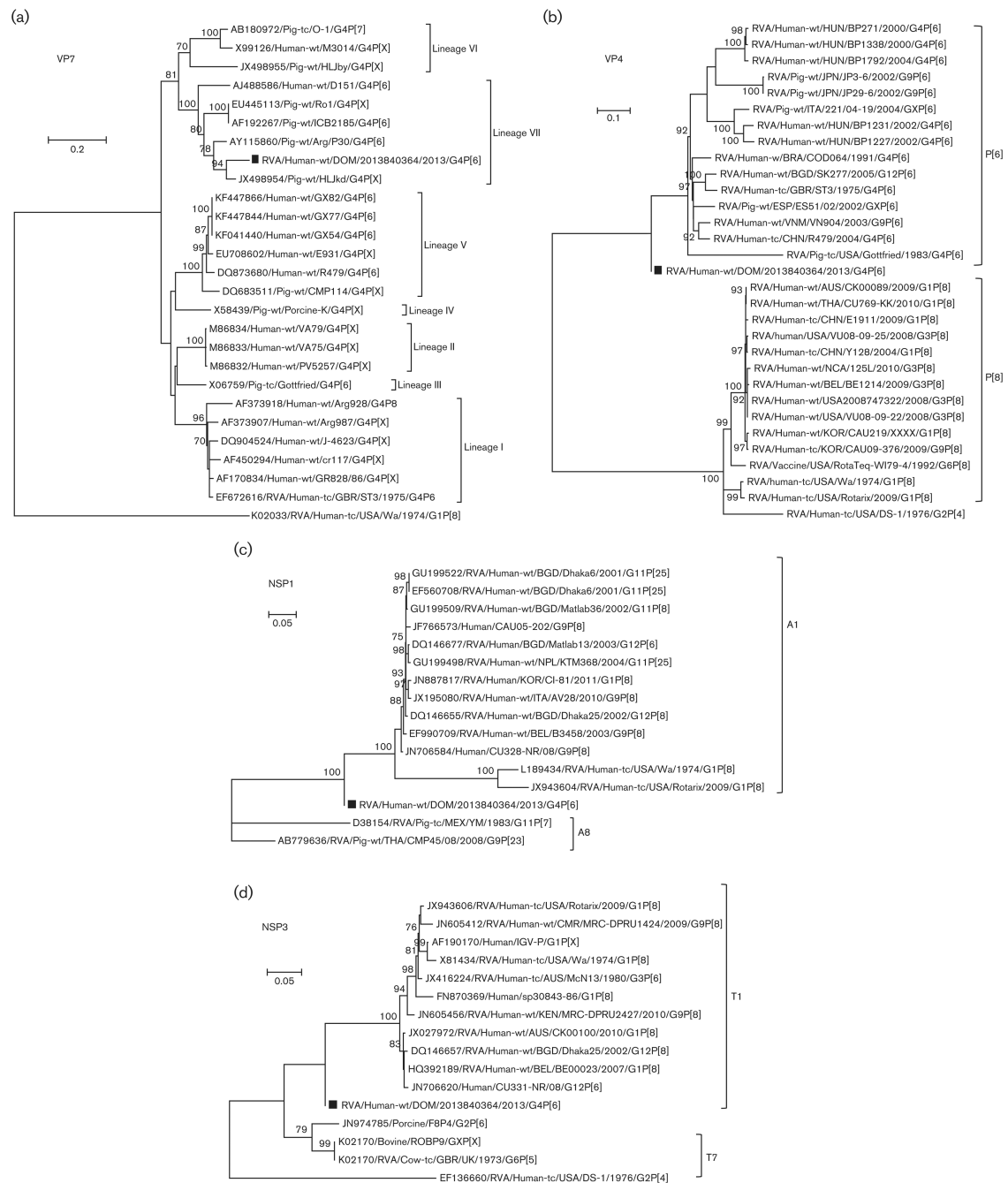
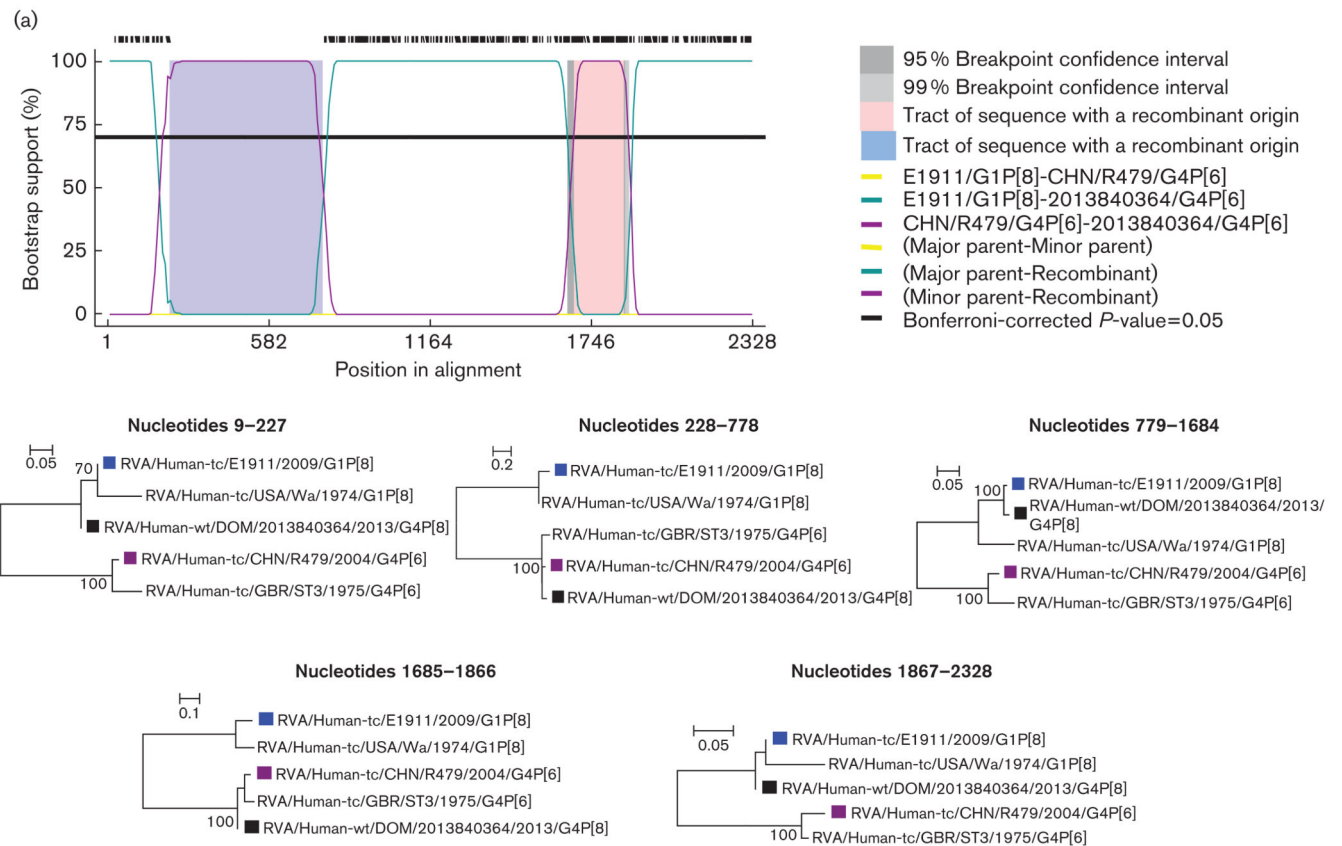
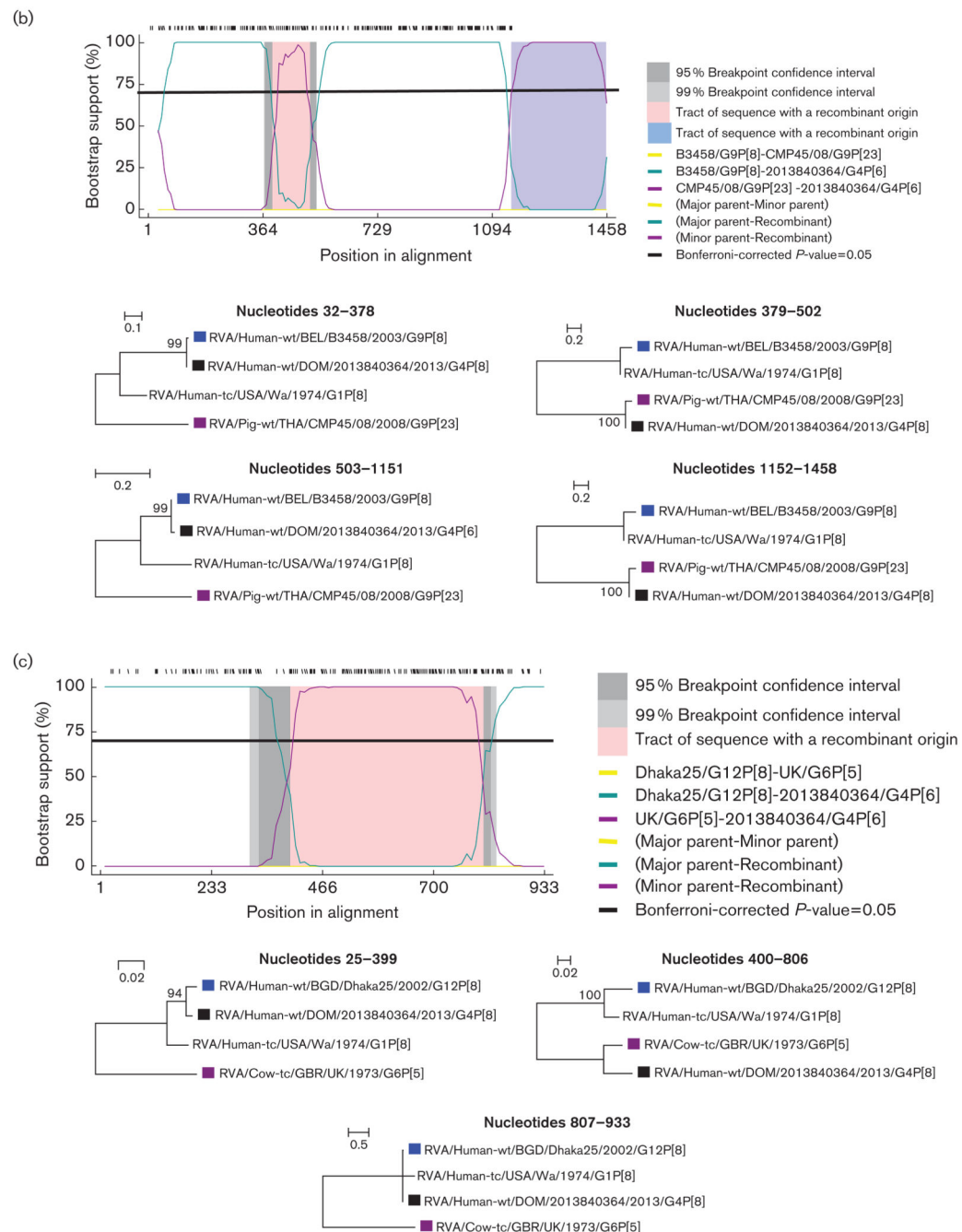


Fig. 1. Phylogenetic trees based on the nucleotide sequences (ORF) of the VP7(a), VP4(b), NSP1(c) and NSP3(d) gene segments. Strain RVA/Human-wt/DOM/2013840364/2013/G4P[6-8_R] is identified by the black filled box. Approximate-likelihood ratio test values >70 are shown adjacent to each node. The GenBank strain names, and G- and P-type associations are shown where available. Each scale bar indicates the number of nucleotide substitutions per site.



**Fig. 2.**

Identification of recombinant VP4, NSP1 and NSP3 genes. (a) Boot-scanning plot of the RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] VP4 gene sequence against VP4 derived from strains E1911/2009/G1P[8] and CHN/R479/2004/G4P[6] is depicted, with region from nucleotides 228 to 778 (purple) and 1685–1866 (pink) highlighted. Plot is modelled on a pairwise alignment with a step size of 20 and 2000 bootstrap replicates. ML trees showing phylogenetic relationships of the recombinant strain 2013840364/2013/G4P[6–8_R], strains E1911/2009/G1P[8] and CHN/R479/2004/G4P[6] in the sequences

prior to the breakpoint, nucleotides 9–227; within the recombined gene segment, nucleotides 228–778 and 1685–1866; and after the breakpoint, nucleotides 779–1684 and 1867–2328. The VP4 genes from strains Wa/1974/G1P[8] and ST3/1975/G4P[6] were used as outgroups. (b) Boot-scanning plot of the RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] NSP1 gene sequence against NSP1 derived from strains B3458/2003/G9P[8] and CMP45/08/2008/G9P[20] is depicted, with region from nucleotides 379 to 502 and 1152–1458 highlighted in pink and purple, respectively. Plot is modelled on a pairwise alignment with a step size of 20 and 2000 bootstrap replicates. ML trees showing phylogenetic relationships of the recombinant strain 2013840364/2013/G4P[6–8_R], strains B3458/2003/G9P[8] and CMP45/08/2008/G9P[20] in the sequences prior to the breakpoint, nucleotides 1–378; within the recombined gene segment, nucleotides 379–502 and 1152–1458; and after the breakpoint, nucleotides 503–1151. The NSP1 gene from strain Wa/1974/G1P[8] was used as an outgroup. (c) Boot-scanning plot of the RVA/Human-wt/DOM/2013840364/2013/G4P[6–8_R] NSP3 gene sequence against NSP3 derived from strains Dhaka25/2002/G12P[8] and UK/1973/G6P[5] is depicted, with region from nucleotides 400 to 806 highlighted in pink. Plot is modelled on a pairwise alignment with a step size of 20 and 2000 bootstrap replicates. ML trees showing phylogenetic relationships of the recombinant strain 2013840364/2013/G4P[6–8_R], strains Dhaka25/2002/G12P[8] and UK/1973/G6P[5] in the sequences prior to the breakpoint, nucleotides 1–399; within the recombined gene segment, nucleotides 400–806; and after the breakpoint, nucleotides 807–933. The NSP3 genes from strain Wa/1974/G1P[8] were used as outgroups.

Table 1

Nucleotide and amino acid percentage identities of complete genome segments of strain RVA/Human-wt/DOM/2013840364/2013/G4P[6-8_R] compared to cognate gene sequence of the closest strains from GenBank database

Gene	Closely related strains	Nucleotide (%)	Amino acid (%)	Genotype	Accession no.
VP1	RVA/Human-wt/NCA/64J/2010/G3P[8]	99.2	99.9	R1	JN129052
VP2	RVA/Human-wt/NCA/64J/2010/G3P[8]	99.9	99.9	C1	JN129052
VP3	RVA/Human-wt/NCA/64J/2010/G3P[8]	98.8	99.4	M1	JN129052
VP4 *	RVA/Human-wt/VNM/VN904/2003/G9P[6]	89.1	90.4	P[6]	EF179118
VP6	RVA/Human-wt/CHN/BJ-CR5317/2008/G9P[8]	99.4	100.0	I1	GU947705
VP7	RVA/Pig-wt/CHN/HLJkd2011/G4P[X]	91.5	93.9	G4	JX498954
NSP1 *	RVA/Human-wt/BEL/B3458/2003/G9P[8]	91.4	89.7	A1	EF990709
NSP2	RVA/Human-wt/ITA/JES11/2010/G9P[8]	99.3	99.7	N1	JX195092
NSP3 *	RVA/Human-wt/BGD/Dhaka25/2002/G12P[8]	90.1	94.2	T1	DQ146657
NSP4	RVA/Human-wt/BGD/Dhaka25/2002/G12P[8]	99.4	99.4	E1	DQ146657
NSP5	RVA/Human-wt/BEL/B4633/2003/G12P[8]	96.8	98.5	H1	EF990709

* Gene segments determined to be recombinant using RDP4 [40, 41] recombination detection software.